Unravelling the molecular mechanisms underlying mitochondrial dysfunction in metabolic diseases

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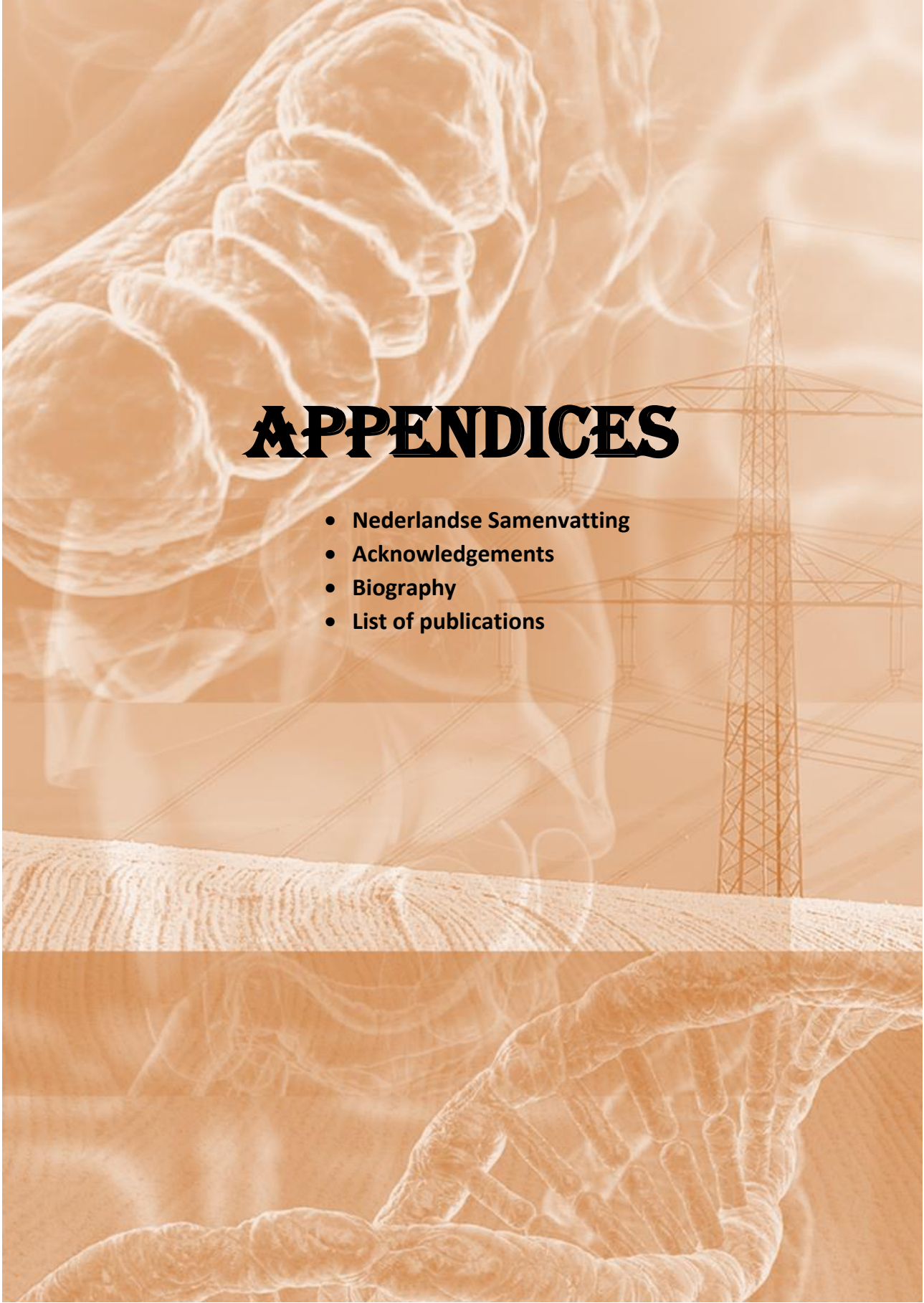
DOI:
10.33612/diss.146092791



APPENDICES

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NEDERLANDSE SAMENVATTING

Ons lichaam heeft energie nodig om goed te kunnen functioneren. In het lichaam wordt deze energie geproduceerd door kleine organellen die mitochondriën worden genoemd. Deze mitochondriën bevatten hun eigen set genetisch materiaal, bekend als mitochondriaal DNA (mtDNA). Afhankelijk van zijn functie bevat elke cel talrijke mitochondriën. In organen die veel energie nodig hebben zoals het hart, de hersenen, de lever en het spierweefsel, zijn meer mitochondriën per cel aanwezig. Cellen hebben energie nodig om verschillende processen aan te sturen. Alles wat een energietekort veroorzaakt, kan de ontwikkeling van zogenaamde stofwisselingsziekten bevorderen.

Niet-alcoholische steatohepatitis (NASH) en myopathie zijn complexe stofwisselingsziekten, beide gekenmerkt door mitochondriale disfunctie, hoewel de onderliggende oorzaken niet goed worden begrepen. Tot voor kort was er een brede discussie over de vraag of het mitochondriale DNA wordt gecontroleerd door epigenetische factoren. Een van deze factoren is DNA-methylatie, de meest bestudeerde DNA-modificatie. De grootste vraag was of het mitochondriaal DNA inderdaad gemethyleerd is en zo ja, wat de relevantie is van deze methylering. In de nucleaire DNA-context wordt methylering binnen bepaalde DNA-gebieden, zoals promotors, geassocieerd met repressie van het gen. Of dit ook geldt voor mitochondriaal DNA blijft echter een veel bediscussieerd onderwerp. Hoewel controversieel, kan de relevantie van door methylering afgeleide veranderingen in mtDNA in relatie tot mitochondriale disfunctie antwoorden bieden op enkele van de onverklaarde oorzaken van metabole ziekten. Dit wordt uitgebreid besproken in **hoofdstuk 2** van dit proefschrift. Dit deel van het proefschrift geeft een overzicht van ziekten die eerder in verband zijn gebracht met differentiële mtDNA-methylering en suggereert epigenetische mechanismen die de expressie van mitochondriale genen kunnen sturen. In **hoofdstuk 3** hebben we geprobeerd de rol van mtDNA-methylatie bij het bevorderen van mitochondriale disfunctie te begrijpen op basis van eerdere studies. Deze studies suggereerden dat methylatie een effect kan hebben op mtDNA-transcriptie en replicatie. Het daaropvolgende debat over het bestaan van mtDNA-methylering wordt ook in de scope van dit proefschrift beschreven om inzicht te geven in de vorderingen die zijn gemaakt op het gebied van mitochondriale epigenetica.

Ook onderzoeken we in dit proefschrift de impact van mtDNA-methylatie op de mitochondriale gezondheid en het metabolisme. Verder wordt in **hoofdstuk 3** en **hoofdstuk 4** mtDNA-methylatie voorgesteld als een oorzaak van mitochondriale disfunctie of een pro-cel-overlevingsmechanisme dat ontstaat als gevolg van de ziekte. In dit opzicht is het moeilijk vast te stellen of mtDNA-methylering een oorzaak is van stofwisselingsziekte of slechts een gevolg van de ziekte. Differentiële DNA-methylatie is eerder beschreven bij NASH-patiënten. In dit proefschrift onderzoeken we of mtDNA-methylering een rol kan spelen bij het bevorderen van mitochondriale

disfunctie. We hebben levercellen ontwikkeld die gemethyleerd mtDNA hebben. Met behulp van dit kunstmatige systeem wilden we onderzoeken of methylatie de mitochondriale ademhaling verstoort, de expressie van mitochondriale genen beïnvloedt en het lipidenmetabolisme verstoort. Bij gebruik van dit technische hulpmiddel lijkt het erop dat methylering van het mtDNA mitochondriale disfunctie veroorzaakt. We suggereren dat mtDNA-methylatie de mitochondriale genexpressie beïnvloedt, wat op zijn beurt de vertaling van eiwitten beïnvloedt die nodig zijn om de elektronentransportketen (ETC) op te zetten. De ETC is vooral belangrijk bij de productie van energie in de vorm van adenosinetrifosfaat (ATP).

Een van de relevante vragen die in **hoofdstuk 3** aan de orde komen, is of mtDNA-methylatie een oorzaak of gevolg is van lipidenophoping in hepatocyten tijdens de progressie van NASH. Verder wilden we inzicht krijgen in hoe mitochondriale genexpressie verandert tijdens de progressie van niet-alcoholische leververvetting (NAFLD) van eenvoudige steatose tot NASH. Eerder is aangetoond dat de lever tijdens de eerste stadia van NAFLD gebruikmaakt van compensatiemechanismen die mitochondriën beïnvloeden om de effecten van het verstoorde metabolisme tegen te gaan. Inzicht in de intracellulaire mitochondriale dynamiek zou licht kunnen werpen op hoe mitochondriale disfunctie wordt bevorderd in NASH, en deze informatie kan van belang zijn bij het bepalen van de therapeutische mogelijkheden waarbinnen zelfs milde interventies de grootste kans op succes zullen hebben.

In **hoofdstuk 4** wordt mtDNA-hypermethylering gepresenteerd als een potentiële biomarker voor een verstoord metabolisme dat zich manifesteert als myopathie. Ook interessant is dat we zien dat myopathiepatiënten verhoogde mRNA-niveaus van SLC25A26 hebben, het gen dat codeert voor de mitochondriale importeur van s-adenosylmethionine (SAM), SAMC. We suggereren dat een verhoogde SAM-import in de mitochondriën aanwijzingen kan geven om de toename in mtDNA-methylatie, zoals waargenomen bij myopathiepatiënten, te verklaren. We concluderen dat niveaus van mtDNA-methylatie bij myopathiepatiënten correleren met ATP-genererend vermogen. Bovendien suggereren we dat mtDNA-methylatie bijdraagt aan een verminderd metabolisme bij myopathie.

In **hoofdstuk 5 en 6** introduceren we koolmonoxide (CO) als een potentiële therapeutische interventie om het herstel van de lever na leverschade te verbeteren. We zien dat CO-werkingsmechanisme het cellulaire energiemetabolisme stimuleert. Lage doses CO beschermen vitale organen zoals hersenen, hart, longen en lever tijdens sepsis, hypoxie en orgaantransplantatie. **Hoofdstuk 5** laat zien dat CO mTOR in hepatocyten activeert en de proliferatie van hepatocyten versterkt door een mechanisme dat de inductie van glutaminolyse in mitochondriën omvat. Door CO geïnduceerde glutaminolyse biedt een energetisch voordeel voor prolifererende cellen en verschuift hepatocyten van een rustige aërobe toestand naar een zeer energetische toestand die de broodnodige energie levert om de regeneratie van de

lever te stimuleren. In dit verband concluderen we dat blootstelling aan CO bijzonder gunstig kan zijn om de regeneratie van de lever te stimuleren.

Bij metabole ziekten zoals NASH, wordt de levensvatbaarheid van hepatocyten beïnvloed door de inflammatoire omgeving, zoals beschreven in **hoofdstuk 6**. De ontstekingsremmende activiteit van CO in onze i*n vitro* NAFLD-modellen biedt een mogelijke krachtige therapeutische interventie voor NASH. We presenteren ook een *in vitro* model met dubbele hit met door vrije vetzuur geïnduceerde lipidenophoping en door tumornecrosefactor-alfa (TNFα) geïnduceerde ontsteking. We laten zien dat TNFα-gemedieerde ontsteking de accumulatie van lipiden in hepatocyten versterkt. TNFα versterkt ook de expressie van Pnpla3, wat een mogelijk mechanisme verschaft voor de invloed van ontsteking op het lipidenmetabolisme. Verhoogde PNPLA3-expressie is geassocieerd met ziekteprogressie bij NAFLD. Vetzuurbelading induceert de expressie van Pnpla3 onder *in vitro*-omstandigheden. **In hoofdstuk 6** zagen we, hoewel CO-behandeling de accumulatie van lipiden in ons *in vitro* NAFLD-model niet verminderde, een significante afname in Pnpla3-expressie en een afname in inflammatie.

Concluderend, epigenetische verandering, met name methylering in het mitochondriale genoom, is een mogelijk mechanisme dat mitochondriale disfunctie bij NAFLD en myopathie bevordert, waardoor de energieproductie wordt beïnvloed. We concluderen ook dat CO de proliferatie van hepatocyten bevordert en de mitochondriale functie verbetert door het mitochondriale energiemetabolisme te verbeteren.

ACKNOWLEDGEMENTS

In my pursuit of knowledge, I took a path that has led me to this moment. I have not walked this path alone and it is my pleasure to acknowledge all who have held my hand and pointed me in the right direction. Firstly, thank you prof **Klaas Nico Faber** for standing with me throughout this PhD period up till now. Thank you for the advice you gave me and for always giving me room to explore over and beyond. You always made it your priority to lighten up the mood in those very long progress meetings we had. When things didn't seem to go the way we expected them to, you never ran out of brilliant ideas. I can never thank you enough because words cannot fully express the extend to which I am grateful. I remember during my first day as a PhD student you told me that you would do everything you can to make sure that I got what I needed to excel. Fast-forwarding to this day I can safely say that you stayed true to your word and you kept your promise. Thank you Klaas Nico.

Many thanks and my utmost gratitude to you prof **Marianne Rots**. Oh Marianne, I am lost for words. I don't even know where to start because we have come a long way. I remember the very first time I met you in 2014. It seems like only yesterday, but it's been 6 years now (still counting….) and you have been with me on this walk along this path that has led us to this moment. Not only did I learn about epigenetics and science but you also gave me important life lessons and advice which I have kept so dearly. I remember the random ice cream invitations during summer and the dinners at your house. You are a wonderful host and I really enjoyed the cozy "gezelligheid" moments we shared in the comfort of your home with your family. Thank you, Marianne. In Shona we say, "Chakanaka chose ngachirumbidzwe." (which can be loosely translated to, "all good things must be acknowledged"). I honestly cannot thank you enough.

This has been a beautiful journey indeed and I have had the privilege of working with so many colleagues who later turned out to be my closest friends. To **Sandra**, **Fabio**, **Violetta**, **Rima** and **Turu**. You guys are like family to me. Thank you for being there for me when I needed you. **Sandra**, I can't seem to find the right words. All I can say is there are so many good memories we have shared along the way. I will always cherish those. Thank you for telling me to take it easy and to relax when I needed to. From the lab, to the office, to weekend dinners with friends, salsa, bachata and then to the gym. **Fabio**, I remember the burritos, the cinnamon rolls and the tasty Mexican cuisine. Thank you for being a part of this journey with me. **Violetta** and **Rima**, I will always cherish the fun moments we had including that time we came out 5th in that pop quiz. First place would have been nice but that's not the most important thing in life…..the moments we shared and memories we made are the most important. **Turu** my buddie, I am always reminded of that time we shared an AirBnB in Germany. With you there was never a dull moment and those were fun times indeed. To my dearest friends **Di** and **Adam**, thank you for the dinner invitations, BBQs and birthday parties. Above all, **Di**, thank you for being my go-to person.

I would like to make special mention of the MDL group. I couldn't have picked a better group to be part of. You guys are amazing. You made my experience worthwhile. Prof **Han Moshage**, prof **Gerard Dijkstra**, **Manon**, **Tjasso**, **Janette**, **Dianne**, **Ali**, **Danial, Shiva, Natalia, Fabio, Turu, Yana, Guido, Ying, Mengfan, Johanna, Raphael, Herson, Alfredo, Floris, Werna** and **Esther**. To **Han**, thank you for the scientific advice you gave me. Besides science, I wish I had more time to convince you that Phill Collins is a better musician, hahaha. To **Ali**, my brother, you were a pillar of support throughout my stay in MDL and you

never tired to give a helping hand whenever you could. To **Natalia**, you are an amazing person to work with and I am glad I had the privilege to work with you. Thank you for assisting me with the seahorse experiments and for going the extra mile whenever I asked for your help. To **Yana**, thank you for offering to do some of my additional experiments when I left Groningen for Zimbabwe. You did it selflessly and for that I am eternally grateful. To **Tjasso**, my dear neighbour, I have so much to thank you for but not enough space to say it all. Thank you for not hiding your enthusiasm when we were working on the CORMs. I absolutely enjoyed working with you. Oh and thank you for the free Sunday tickets to watch FC Groningen. I really enjoyed those Sunday afternoons all thanks to you **Tjasso**. To **Manon**, "Ik heb een vraag." Yes I still have a lot of questions lined up for you, hahaha. Thank you for being there when I needed you and for answering all my questions even though they were so many of them. To **Jannette**, thank you for assisting me with the oil red stainings. That was quite a lot of work and you had patience to scan all the slides even at your own time. I really appreciate the help you gave me. To **Dianne**, my dear neighbour whom I had no border disputes with (unlike the border disputes I had with **Tjasso**, hahaha). Thank you for your assistance with the DNA/RNA extraction kits. You made my life so much easier and for that I appreciate the help you gave me.

I would also like to make special mention of the EGE group. **Marcel, Jelleke, Anita, Pytrick, Marloes, Desiree, Dandan, David, Julio** and **Fabian**. Not forgetting **Monique**, thank you for all the assistance you gave me and for setting the pace in the mtDNA methylation project. To **Marloes, Desiree, Dandan** and **Monique**, outside the lab I remember the dinners we had. Those were fun times and I really enjoyed your company. I am also reminded of the time we went to the military escape room at the Grote Markt….to cut the long story short, we made it out in less than 30 minutes. If I could turn back the hands of time I would repeat those moments over and over again. To **Marloes**, my friend, colleague and travel partner. I'm reminded of the Italian road trip we had after summer school from Jesi to Bologna. That was the most memorable time and I am glad to have shared those moments with you. Oh, and thank you for teaching me everything I know about Syrian hamsters. To **Desiree**, thank you for being the one who knew where everything was (if I needed something I knew you knew where to find it) and for always beaming a smile that lightened up your surroundings. To **Jelleke**, thank you for giving me my first cell culture practical lesson and for being there when I needed you. **Anita**, you are a star. Thank you for helping me out with sorting out my stuff in the freezers and for always giving me a hand when I needed one.

To my office mates, **Sandra, Joanne, Marleen, Herson, Mengfan, Ying, Josie, Rumei** and **Marcela**. You guys rock. It was a real pleasure to work with you all. **Joanne**, I still remember ctrl-Shift-T. Thanks to you it made copying and pasting data in graphpad so much easier. Thank you for all the assistance you gave and for attempting to answer some of the scientific questions I posed at you and also for helping me with my Dutch summary. You were, and still are eager to give a helping hand whenever you can.

From the Pediatrics lab I would like to thank **Joanne, Mirjam**, **Niels, Aycha, Angelica, Jan Freark, Ana, Marleen, Angela, Tim, Onne** and **Irene.** I remember the Friday after work drinks, "Vrijmibo." Those were quite memorable times.

From the lab of Torsten Plosch, many thanks go to **Rijst Nynke**, **Violetta** and **Dorieke**. Thank you **Rijst Nynke** for assisting with cleaning and general maintenance of the Q48

pyrosequencing machine. **Violetta**, I always knew I could count on you when it came to troubleshooting pyrosequencing errors.

From the laboratory of metabolic diseases, I would like to thank dr **Klary Niezen-Koning** for the unwavering technical support and assistance with Chapter 4 of this thesis. **Klary** thank you for going through all the hustle of finding myopathy patient samples and for making this exciting project come to life. Many thanks to **Crista, Dilemin (Lemon)** and **Kevin** for assisting me with the myopathy project. I couldn't have done it without you guys.

Many thanks go to Pastor **John, Clara, Michael, Margret** and **Ria**. Pastor **John** thank you for being my brother in the faith, my mentor and my friend. You welcomed me into your home and made me a part of your family. You made me feel at home in Groningen and for that I am exceedingly grateful. I consider Groningen my second home. Many thanks also go to my father in the faith, Apostle **Josphat Sithole** for the prayers and encouragement you gave me. I am grateful that you kept me in check.

I would like to thank my parents **David** and **Getrude** for always motivating me and for believing in me. Thank you, Mom and Dad. Your prayers for me were answered. God has been faithful. To my siblings, **Teclar, Munya** and **Fadzi**, you guys are truly amazing. The calls you made whilst I was so far away brought me so close to home. Coming back to Zim was always something I looked forward to because I knew I was coming home to a family that loves me.

Last but certainly not least, I would like to thank my dearest beloved wife **Chiedza** who has stood with me and endured the long wait while I was pursuing my studies. **Chie** I am so grateful for you and for the man you have made me become. You kept me sane during times when I had so much pressure mounting on me. You kept encouraging me and telling me to aim for the stars. Above all you made me believe in myself. Thank you for being part of this journey with me. To my son **Ayden Anesu**, I want you to know that you have given me a new purpose in life.

I have learnt over the years that life is happening now. I have learnt to live and cherish every moment because that's when life is happening. I had a great experience both inside and outside the lab. In the midst of it all, every experience was never without its highs and lows. With every challenge that came my way I found someone to help me manoeuvre through the difficult parts. For that I am eternally grateful for everyone who was part of my life during this PhD journey that has finally come to an end. Now taking the next step.

Truly, God has been faithful to me and He has led me this far.

BIOGRAPHY

Archibold Mposhi was born on the 22nd of May 1988 in Marondera, Zimbabwe. He attended his high school at Bernard Mzeki College in Marondera where he majored in Mathematics, Biology and Chemistry. Upon successful completion of his pre-university studies Archie's passion for biology led him to undertake an undergraduate bachelor's degree in Biotechnology at Chinhoyi University of Technology. In 2012 he graduated top of his class with distinction. In 2013, he was granted the Abel Talesman Grant and he was also selected for the Topmaster in Medical and Pharmaceutical Drug Innovation at the University of Groningen in the Netherlands. During this period, he did his internships in the labs of Prof dr Marianne Rots (epigenetics) and Prof dr Klaas Nico Faber (hepatology and gastroenterology), both who were pivotal in establishing his career in science. In 2015 he completed his masters and graduated with distinction, *cum laude*. In the same year he was awarded the Graduate School of Medical Sciences PhD grant to conduct a research topic of his choice. Arche's interest in hepatology and epigenetics set the basis for his PhD topic of choice, which focused on the role of mitochondrial dysfunction in metabolic diseases under the guidance of Prof dr Klaas Nico Faber and Prof dr Marianne Rots. Archie also presented his work at various forums in the Netherlands, Germany and Italy. Upon completion of his PhD studies, he would like to pursue the field of mitochondrial (epi-)genetics to fully understand how these tiny organelles shape our destiny.

LIST OF PUBLICATIONS

Mposhi, A., van der Wijst, M.G.P., Faber, K.N and Rots, M.G. (2018) Unravelling the Effects of Mitochondrial DNA Methylation on Hepatic Energy Metabolism. Lifestyle Genomics. 11:19-38 [Abstract]

Mposhi, A., van der Wijst, M.G.P., Faber, K.N and Rots, M.G. (2017) Regulation of mitochondrial gene expression, the epigenetic enigma. Frontiers in Bioscience. 22:1099-1113.

van der Wijst, M.G.P., Huisman, C., **Mposhi, A**., Roelfes, G. and Rots, M.G. (2015) Targeting Nrf2 in healthy and malignant ovarian epithelial cells: Protection versus promotion. Molecular Oncology. 9(7):1259

SHADOWS IN THE LIGHT

Do my eyes deceive me?

Is this a tree I see,

Or is it its shadow I see with glee?

Moving slowly by the day and vanishing by night.

Moving closer,

I can touch it,

I can feel its rough trunk

I can smell its fresh leaves.

Surely this must be a tree

- *Archibold Mposhi* –